

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶: A61K 31/22	A1	(11) International Publication Number: WO 00/27386 (43) International Publication Date: 18 May 2000 (18.05.00)
(21) International Application Number: PCT/IT98/00318 (22) International Filing Date: 11 November 1998 (11.11.98) (71) Applicant (for all designated States except US): SIGMA-TAU INDUSTRIE FARMACEUTICHE RIUNITE S.P.A. [IT/IT]; Viale Shakespeare, 47, I-00144 Rome (IT). (72) Inventor; and (75) Inventor/Applicant (for US only): CALVANI, Menotti [IT/IT]; Via Longanesi, 14, I-00146 Rome (IT). (74) Common Representative: SIGMA-TAU INDUSTRIE FARMACEUTICHE RIUNITE S.P.A.; Viale Shakespeare, 47, I-00144 Rome (IT).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>In English translation (filed in Italian).</i>
(54) Title: USE OF PROPIONYL CARNITINE FOR THE MANUFACTURE OF A MEDICAMENT FOR INHIBITING SMOOTH MUSCLE CELL PROLIFERATION (57) Abstract The use of propionyl L-carnitine for the preparation of a medicament having inhibiting activity of the proliferation of smooth muscular cells of vascular wall is herein disclosed. Said medicament is useful for the treatment of vascular pathologies, such as atherosclerosis, hypertension, pulmonary hypertensions, restenosis after angioplasty.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

USE OF PROPIONYL CARNITINE FOR THE MANUFACTURE OF A MEDICAMENT FOR INHIBITING SMOOTH MUSCLE CELL PROLIFERATION

The present invention relates to the use of propionyl L-carnitine
5 and the pharmaceutically acceptable salts thereof for the preparation
of medicaments useful in the treatment of blood vessel pathologies.

Background of the invention.

A number of studies demonstrated that cell proliferation plays a
pivotal role in atherosclerosis, hypertension pathogenesis and
10 restenosis after angioplasty or coronary stenting (Ross, 1976;
Schwartz, 1990).

Many experimental studies, carried out on human
atherosclerotic plaques, demonstrated that cell proliferation is a
determining phenomenon both in the early phases and in the
15 progression of the plaque.

Further, proliferation of smooth muscle cells, which migrated to
intima from vascular tunica media, represents cell basis of coronaric
restenosis after rivascularization processes through angioplasty or
dilatation by means of a stent.

20 This drawback is the major limit to the application of
percutaneous rivascularization in patients affected by acute coronary
syndromes, since it is responsible for about 40% of post-surgical
failures (Holmes et al., 1984).

Therefore, making available substances capable of controlling the proliferation of smooth muscular cells of vessel wall is a goal of primary importance in the prevention of restenosis after angioplasty, as the proliferative phenomenon occurs in a determined timed
5 corresponding to the first weeks following the intervention.

Proliferation control in experimental atherosclerotic lesions has been obtained with cytostatic drugs, such as etoposide (Llera-Moya et al., 1992), with steroid hormones (Cavallero et al.; 1971; 1973; 1975; 1976), progestinic hormones (Spagnoli et al., 1990), dexamethasone
10 (Asai et al., 1993).

Smooth muscle cell proliferation is also inhibited by calcium antagonist substances due both to a decrease of DNA synthesis, such as in case of verapamil (Stein et al., 1987) and to the interference in second messenger systems (cAMP), as demonstrated for nifedipine
15 (Cheung et al., 1987).

The treatment with ACE-inhibitors resulted in the control of the growth of intima thickening (Powell, 1989).

Other in-vitro studies evidenced an antiproliferative effect on cultured smooth muscular cells of rat aorta given by simvastatine, a
20 HMG-CoA reductase inhibitor, used as hypolipidemic agent (Corsini et al., 1991). Further, some substances having triglyceridemia lowering effect, such as fibrates, showed to be able to prevent the progression of atherosclerotic lesions in the human (Olsson et., 1990).

In a manner similar to what observed in neoplasia (Kerr, 1994), phenomena of population decrease are observed to occur together with cell proliferation in atherosclerotic population and/or in intima thickening (Gabbiani, 1995), thus suggesting that highly proliferative
5 cells go toward apoptosis and that modulation of the latter plays an important role in atherosclerotic lesion genesis.

Using apoptosis inducing substances bears the risk to provoke a generalised phenomenon, with possible side effects, which can be even very severe, such as in the case of stem cells.

10 It has now been found that propionyl L-carnitine, thanks to its unexpected proapoptotic effect, is endowed with a specific action of control on smooth muscular cells of vessels.

Abstract of the invention.

It is an object of the present invention the use of propionyl L-
15 carnitine and the pharmacologically acceptable salts thereof for the preparation of a medicament having inhibiting activity on smooth muscular cells of blood vessel walls.

The most important and surprising advantage of the present invention is that the administration of propionyl L-carnitine does not
20 imply toxic effects on bone marrow and in gut, which have a good production of blood cellular elements and a very good turnover of intestinal mucosa cells, respectively. This and other aspects of the

present invention will be illustrated in detail, also by means of examples.

Detailed description of the invention.

The present invention is based on the application of the
5 discovery that propionyl L-carnitine (hereinafter PLC) induces the
phenomenon of programmed death (apoptosis) in the cells. This effect
allows the treatment of blood vessel pathologies based on the
proliferation of smooth muscular cells of vessel walls, such as
pulmonary hypertension, atherosclerosis, hypertension, restenosis after
10 angioplasty.

Accordingly, a first aspect of the present invention relates to the
use of propionyl L-carnitine and the pharmacologically acceptable salts
thereof for the preparation of a medicament having inhibiting activity
on the proliferation of smooth muscular cells of vessel walls.

15 A further object of the present invention relates to the use of
propionyl L-carnitine and the pharmacologically acceptable salts
thereof for the preparation of a medicament useful for the treatment of
atherosclerosis.

Another object of the present invention is the use of propionyl L-
20 carnitine and the pharmacologically acceptable salts thereof for the
preparation of a medicament useful for the treatment of hypertension.

A fourth object of the present invention is the use of propionyl
L-carnitine and the pharmacologically acceptable salts thereof for the

preparation of a medicament useful for the treatment of pulmonary hypertension.

Still another aspect of the present invention is the use of propionyl L-carnitine and the pharmacologically acceptable salts thereof for the preparation of a medicament useful to prevent restenosis after angioplasty.

The medicament according to the present invention can be obtained admixing the active ingredient (propionyl L-carnitine or a pharmacologically acceptable salt thereof) with excipients suitable for formulation of compositions intended for enteral administration (in particular the oral one) or parenteral administration (in particular through intramuscular or intravenous route). All such excipients are well known to persons skilled in the art.

As pharmaceutically acceptable salt of propionyl L-carnitine, it is intended any salt thereof with an acid which does not give rise to unwanted side effects. These acids are well known to the pharmacologists and to the experts of pharmaceutical technology.

Non-limiting examples of said salts are chloride, bromide, orotate, acid aspartate, acid citrate, acid phosphate, fumarate and acid fumarate, lactate, maleate and acid maleate, acid oxalate, acid sulphate, glucose phosphate, tartrate and acid tartrate.

Some examples of formulations in the form of unitary dosages are given.

(a) **Formulation for tablets**

A tablet contains:

Active ingredient

propionyl L-carnitine HCl mg 500

Excipients

Microcrystalline cellulose mg 54.0

Polyvinylpyrrolidone mg 18.0

Crospovidone mg 30.0

Magnesium Stearate mg 15.0

Fumed silica mg 3.0

Hydroxypropylmethylcellulose mg 10.0

Polyethylene glycole 6000 mg 2.5

Titanium dioxide mg 1.8

Methacrylate copolymer mg 8.3

Talcum (trivalentilated) mg 2.4

(b) **Formulation of intravenously injectable bottles**

A bottle contains:

Active ingredient

Propionyl L-carnitine HCl mg 300

Excipient

Mannitol mg 300

A solvent vial contains:

Sodium acetate 3·H₂O mg 390

Water for injectable F.U. q. s. to ml 5

The medicament prepared according to the present invention will be administered in the form of pharmaceutical composition, which can be prepared according to the general common knowledge of the person skilled in the art.

Depending on the administration route appropriately chosen, oral, parenteral or intravenous; the pharmaceutical composition will be in the suitable form.

Examples of pharmaceutical compositions, wherein the medicament according to the present invention is comprised, are the solid or liquid oral forms, such as tablets, all types of capsules, pills, solutions, suspensions, emulsions in the form of unitary or divided doses, syrups, ready-to-use or extemporaneous drinkable unit doses. Other examples are parenteral forms, injectable forms for intramuscular, subcutaneous or intravenous administration. Controlled or programmed release forms are also appropriate.

Dosages, posology and general therapeutic regimen will be determined by the physician according to his knowledge, patient's conditions and the pathology to be treated.

The association, whether co-administered in the same medicament or separately (at the same time or subsequently) of PLC with other active ingredients is also comprised in the present invention.

In a first preferred embodiment, the present invention relates to restenosis after angioplasty.

According to this first preferred embodiment, the pharmacological dose of PLC is such as not to exceed hematic
5 concentration of 100 mM.

The following example further illustrate the invention.

EXAMPLE

Wistar male rats, weighing between 270 and 290 mg, were used for the experiments. The rats were anaesthetised with Nembutal i.p.
10 (35 mg/kg body weight) and the thoracic portion of aorta was submitted to endothelium mechanical removal with Fogarty 2F balloon probe (Baxter USA), according to the Baugartner and Studer method(1966) with minor modification (Orlandi 1994). The animals were randomized into 5 groups, each group is reported in Table 1.

15 Two groups were subjected to pharmacological treatment with propionyl L-carnitine (PLC, 120 mg/Kg p.c. die), one group was treated with an ACE-inhibitor (Enalapril, 1 mg/Kg p.c. die); the two remaining groups were the control. Moreover, some non-balloonized animals were used as blanks.

Table 1

Final number of Wistar rats	Treatment	Duration (days)
7	de-endothelialization + PLC	3
7	de-endothelialization	3
8	de-endothelialization + ACE-antagonist	15
8	de-endothelialization + PLC	15
8	de-endothelialization	15
5	blanks	-

The animals were sacrificed 3 and 15 days after de-
 5 endothelialization. Two hours before sacrifice, all the rats received i.v.
 a Bromodeoxyuridine solution (BrDU) (45 mg/kg body weight) in order
 to verify cell proliferation. One hour before sacrifice, some randomly
 selected animals received 1 ml Blue Evans (1% in 0.9% NaCl solution)
 in order to evaluate the degree of aorta disruption.

10 At sacrifice, the animals were anaesthetised with i.p. Nembutal
 and perfused, after washing with isotonic saline containing 3%
 Dextran 70, with buffered formalin for 20 minutes. Aortae were
 isolated, slightly washed in saline and dissected longitudinally.
 Carotid, heart and small intestine were also excised. All the organs
 15 were post-fixed in the same fixative for 24 hours at room temperature.
 Some aortic fragments were used for electronic microscopy. Aortae
 were rolled up and included in paraffin. Serial sections having 5 μ m

thickness were stained with Hematoxylin-Eosine, Verhoeff-Van Gieson and Movat's pentachromic and used for morphologic and morphometric studies.

In some non-perfused animals, fragments of aortic tissue were frozen in liquid nitrogen for the determination of tissular carnitines and for subsequent studies of molecular biology.

Immunohistochemical staining

In order to put in evidence proliferating cells in damaged arteries, serial sections in paraffin of aortae were deparaffined, rehydrated, immersed in a 3% H_2O_2 solution for 20 minutes and incubated with trypsin (0,05 M in Tris-HCl, pH 7.6) at 37°C. After that, sections were treated with 2N HCl at 37°C for 30 minutes, washed with 0.1 M sodium tetraborate for 10 minutes, incubated with normal horse serum (Vector) and subsequently with anti-BrDU monoclonal antibody (Ylem) for 1 hour. The preparates were then reacted with biotilinated anti-mouse IgG (Vector) and the Streptoavidine-ABC-POD complex (Ylem).

The reaction was evidenced by using diaminobenzidine (DAB) as final chromogen. The count of positive nuclei for BrDU was made on the total number of nuclei. Such count was blind-made by two researchers separately. The difference between the two counts was always lower than 0.5%.

All data were analysed with the T Student's test. The differences between the groups were considered to be significant for $P < 0.05$.

Morphometric analysis

The entity of intima thickening after 15 days was evaluated on
5 Verhoeff-Van Gieson stained sections, using a grid overlapped on the image, consisting of 400 points, 1 cm from each other.

The analysis was made on hystological preparates through a Hamamatsu C3077 camera controlled by a Hamamatsu DVS 3000 image analyser and connected to a Polyvar-Reichert microscope.
10 Morphometric evaluation was made at X116 magnification. The following parameters were evaluated a) relative volume of intima referred to arterial wall; b) relative volume of tunica media referred to arterial wall, by counting the overlapping points on the intima and mean tunica.

15 3-12 aorta sections were used at different level for each animal. This number was a function of the structure sizes, according to Sach's formula, showing the number of fields necessary to obtain a statistically significant sample.

Ultrastructural studies

20 Small aorta samples were selected for electronic microscopy. Aortae were post-fixed in osmium tetroxide and embedded in EPON 812. Ultra thin sections were stained with uranyl acetate followed by

lead citrate and examined using a Hitachi H-7100 FA transmission electronic microscope.

Tissular and plasma carnitine assay

2-3 ml of blood samples were withdrawn from each animal
5 before mechanical de-endothelialization and at the time of sacrifice. Plasma was separated by centrifugation (300 rpm) for 20 minutes and frozen for plasmatic carnitine assay according to the Pace et al. method.

Aorta wall samples were withdrawn from some non-perfused
10 animals, randomly selected from each group, frozen in liquid nitrogen and stored at -80°C for the carnitine assay, according to the above Pace et al. reference.

RESULTS

Ultrastructural studies

15 Small aorta samples were selected for electronic microscopy. Aortae were post-fixed in osmium tetroxide and embedded into EPOC 812. Ultra thin sections were stained with uranyl acetate followed by lead citrate and examined through a Hitachi H-7100 FA transmission electron microscope.

20 Tissular and plasmatic carnitine assay

2-3 ml blood samples were withdrawn from each animal before mechanical de-endothelialization and just before sacrifice. Plasma was

separated by centrifugation (3000 rpm) for 20 minutes and frozen for the plasmatic carnitine assay according to the Pace et al. method.

Samples of aortic wall were taken from some non-perfused animals, randomly selected from each group, frozen in liquid nitrogen and kept at -80°C for the assay of tissutal carnitines according to the above-mentioned Pace et al. method.

RESULTS

L sion morphology

3 days after the mechanical lesion, rat aortae did not show significant hystological alterations, except the lack of endothelial cell coating.

15 days after, remodelling of arteria could be observed for the presence of an intima thickening (or neointima), consisting in round or lengthened cells immersed in abundant extracellular matrix. Immune hystochemical study put in evidence in particular the presence of abundant smooth muscular cells (SMC) inside neointima.

Studies on proliferation

a) **3 days after de-endothelialization:** the count of anti-BrDU staining positive nuclei showed substantial differences between the two groups examined. Quantitative - analysis (Table 2) puts in evidence that the number of BrDU-positive nuclei is significantly lower in the tunica media in the PLC-treated animals, with respect to controls (59.3% reduction against control, $p < 0.02$). In both

groups the distribution of BrDU-positive nuclei is more concentrated in the lumen portion of mean tunica with respect to the adventitia portion, with a 2:1 ratio.

- b) **15 days after de-endothelialization:** Table 3 shows that in each group the proliferation index of SMCs is significantly higher (p<0.001) in the intima with respect to the tunica media. No significant differences are observed in the number of BrDU-positive nuclei, in the intima and tunica media, by comparing PLC, Enalapril and control animals.

10 **Morphometric analysis**

As described in Table 3, after 15 days from endothelial lesion, the intima relative volume is significantly lower, both in the PLC-treated (31.11% reduction against control, p<0.02) and ACE-antagonist-treated (26.14% reduction against control, p<0.01) animals against control animals.

Table 2

In-vivo treatment with propionyl L-carnitine (PLC) on the proliferation of smooth muscle cells of rat aorta after mechanical de-endothelialization: percentage of proliferating cell nuclei (anti-bromodeoxyuridine positive) after 3 days (\pm s.e.m)

	interval	positive nuclei/ total nuclei %	Reduction %	Difference
Mean tunica Control animals	3 days	6.36 \pm 1.27		
Tunica media PLC treated animals	3 days	2.59 \pm 0.56	59.3	P<0.02

Tabl 3

In vivo treatment with propionyl L-carnitine (PLC) and with the ACE-antagonist Enalapril on the proliferation of smooth muscular cells of rat aorta after mechanical de-endothelialization: percentage of proliferating cells (anti-bromodeoxyuridine positive) and percentage ratio between intima volume and aorta wall volume after 15 days (\pm s.e.m.) (preliminary results).

	interval	+ Nuclei/total nuclei %	Intima volume/ wall	Reduction (% to CTR)
Intima tunica, control animals	15 days	2.65 ± 0.44 (a)	29.73 ± 1.54	
Intima tunica, PLC treated animals	15 days.	1.99 ± 0.32 (b)	20.48 ± 2.73 (d)	31.11
Intima tunica, enalapril treated animals	15 days	2.43 ± 0.39 (c)	21.96 ± 1.20 (e)	26.14
Tunica media, control animals	15 days	0.24 ± 0.05		
Tunica media, PLC treated animals	15 days	0.24 ± 0.09		
Tunica media, enalapril treated animals	15 days	0.27 ± 0.09		
Tunica media, non peeled animals		0.24 ± 0.05		

(a) intima vs tunica media: $p < 0.0001$; (b) intima vs tunica media: $p < 0.0001$; (c) intima vs tunica media: $p < 0.001$; (d) intima vol./wall vs controls: $p < 0.02$; (e) intima vol/wall vs controls: $p < 0.01$

EFFECT OF PROPIONYL L-CARNITINE IN THE CONTROL OF PROLIFERATION/APOPTOSIS.

In vitro experiments were carried out to evaluate the effect of propionyl L-carnitine (PLC) on smooth muscular cells (SMC) isolated from aortae of spontaneously hypertensive rats (SHR) and, as control, on SMC isolated from normotensive rats (WKY).

These in vitro studies evidenced that PLC, when administered during culture exponential growth phase, reduces cell growth, evaluated as cell number/ml, as well as DNA synthesis, evaluated through incorporation of tritiated thymidine (Tab. 4 and 5).

Table 4: cell number/ml at culture days 2, 3, 4 and 6

	Day 2	Day 3	Day 4	Day 6
SHR CTRL	$5 \cdot 10^4 \pm 2$	$15 \cdot 10^4 \pm 3$	$28 \cdot 10^4 \pm 7$	$42 \cdot 10^4 \pm 7$
SHR PLC	$7 \cdot 10^4 \pm 4$	$4 \cdot 10^4 \pm 2$	$7 \cdot 10^4 \pm 4$	$20 \cdot 10^4 \pm 5$
WKY CTRL	$4 \cdot 10^4 \pm 1$	$2 \cdot 10^4 \pm 2$	$7 \cdot 10^4 \pm 2$	$13 \cdot 10^4 \pm 4$
WKY PLC	$3 \cdot 10^4 \pm 2$	$4 \cdot 10^4 \pm 2$	$6 \cdot 10^4 \pm 2$	$8 \cdot 10^4 \pm 3$

Table 5: tritiated thymidine incorporation at culture day 6

	$\mu\text{Ci}/\mu\text{gDNA}$
SHR CTRL	2.99 E-05
SHR PLC	1.81 E-05
WKY CTRL	2.1 E-05
WKY PLC	2.56 E-05

As a further characterisation of smooth muscular cells in the presence of PLC, the percentage of apoptotic cells was measured both in basal conditions and in oxidative stress conditions. Apoptosis evaluation was carried out by counting the number of apoptotic cells present on a total of 1000 cells, after specific DNA staining with Hoechst 33258. The results of this experiment demonstrated that, in SHR cultures, PLC determines a significant increase of apoptosis percentage in basal conditions and that this increase is more evident under stress conditions.

In WKY cultures, apoptosis percentage is negligible (tab. 6)

Table 6: apoptotic cell percentage in basal conditions and under oxidative stress.

	Basal conditions	Oxidative stress
SHR CTRL	0	2
SHR PLC	2	10
WKY CTRL	0	0
WKY PLC 2	0	2

The behaviour observed in SHR smooth muscular cells might be in some way related to the deregulated expression of c-myc, which characterises spontaneously hypertensive rats (Negoro et al., 1988). Moreover, it was observed that c-myc actively cooperates in inducing

apoptosis subsequently to a proliferation stop (Bennet et al., 1993; Bissonette et al., 1993), accordingly the data shown above suggest that PLC anti-proliferative effect may be related to an interference with DNA replication.

References:

- Spagnoli L.G. Giorn. Arterioscl. 1983; 8: 117-145
- Asai K, Funaki C, Hayashi T. et al. Arterioscl. Thromb. 1993; 13: 892-899
- 5 Baumgartner H.R., Studer A, Pathol. Microbiol. (Base) 29: 393-405, 1966
- Bennet M.R., Evan G.I., Newby A.C., Circ. Res. 1994; 74:525-536
- Bissonette R.P., Shi Y., Mahboubi A., Glynn J.M. and Green D.R, Curr. Commun. Cell. Mol. Biol. 1993
- 10 Bonanno E., Ghibelli L., Coppola S., Spagnoli L.G., International Conference on Cell Death in Human Pathology. Lecce, June 22-25, p. 54, 1995
- Bouchaton-Piallat M.L., Gabbiani F., Desmouliere A. and Gabbiani G. Am. J. Pathol. 146: 1059-1064, 1995
- 15 Cavallero C., De Lellis C., Di Tondo U. et al. In: Cavallero C., editor. The arterial wall in atherogenesis. Padova: Piccin Medical Book, 1975: 25-42
- Cavallero C., Di Tondo U. Mingazzini L. et al., Atherosclerosis 1973; 17: 49-62
- 20 Cavallero C., Di Tondo U., Mingazzini P. et al. Atherosclerosis 1976; 25: 145-152
- Cavallero C., Turolla E. and Ricevuti G., Atherosclerosis 1971; 13: 9-20

- Cheung W.T., Shi M.N., Young J.D. et al., *Biochem. Pharmacol.* 1987; 36: 2183-2189
- Clowes A.W., Schwartz S.M., *Cir. Res.* 56: 139-145, 1985
- Corsini A., Raiteri M., Soma M. et al., *Pharmacol. Res.* 1991; 23: 173-
5 180
- Curi R., Bond J.A., Calder P.C. and Newsholme E.A., *Gen. Pharmac.* 1993; 24, 591-597
- Holmes D.R.J., Vliestra R.E., Smith H.C., *Am. J. Cardiol.* 1984; 77C-81C.
- 10 Kerr J.F.R., Winterford C.M., Harmon B.V., *Cancer* 73: 2013-2026, 1994
- Llera-Moya M., Rothblat G.H., Glick J.M. et al., *Arterioscler. Thromb.* 1992; 12: 1363-1370
- Mauriello A., Sangiorgi G., Orlandi A., Schiaroli S., Pertumo S.,
15 Spagnoli L.G.,
- Negoro N., Inariba H., Inoue T., Kanayama Y., Takeda T.
- Olsson A.G., Ruhn G., Erikson U., *J. Int. Med.* 227: 381, 1 990
- Orlandi A., Ehrlich H.P., Ropraz P. et al., *Arterioscler. Thromb.* 1994; 14: 982-989
- 20 Orlandi A., Ropraz P. and Gabbiani G., *Exp Cell Res* 1994; 214: 528-536
- Powell J.S., Clozel J-P., Muller R.K.M., Kuhn H., Hefti F., Hosang M., Baumgarner H., *Science* 245: 186-188, 1989

- Ross R., Glomset J.A., N. Engl. J. Med. 295: 369-377, 1976
- Schwartz S.M., Heimark R.L., Majesky M.V., Physiol. Rev. 70: 1177-1209, 1990
- Spagnoli L.G., Giorn. Arterioscl. 1983; 8: 117-145
- 5 Spagnoli L.G., Orlandi A., Marino B., Mauriello A., De Angelis C.,
Ramacci M.T., Atherosclerosis 1995; 114, 29-44
- Spagnoli L.G., Orlandi A., Marino B. et al., Atherosclerosis 1995; 114:
29-44
- Spagnoli L.G., Orlandi A., Mauriello A., et al., Pathol. Res. Pract. 1992;
10 4-5: 637642
- Spagnoli L.G., Orlandi A., Mauriello A. et al. Atherosclerosis 1991; 89:
11-24.
- Spagnoli L.G., Palmieri G., Mauriello A. et al., Atherosclerosis 1990;
82: 27-36
- 15 Spagnoli L.G., Sambuy Y., Palmieri G. et al., Artery 1985; 13: 187-198
- Stein O., Halperin G., Stein Y., Arteriosclerosis 1987; 7: 585-592

CLAIMS

1. Use of propionyl L-carnitine and pharmaceutically acceptable salts thereof for the preparation of a medicament having inhibiting activity of the proliferation of smooth muscular cells of vascular wall.
5
2. Use according to claim 1, wherein said medicament is useful for the treatment of atherosclerosis.
3. Use according to claim 1, wherein said medicament is useful for the treatment of hypertension.
- 10 4. Use according to claim 1, wherein said medicament is useful for the treatment of pulmonary hypertension.
5. Use according to claim 1, wherein said medicament is useful for the prevention of restenosis after angioplasty.
- 15 6. Use according to anyone of claims 1 to 5, wherein said salt of propionyl L-carnitine is selected from the group consisting of chloride, bromide, orotate, acid aspartate, acid citrate, acid phosphate, fumarate and acid fumarate, lactate, maleate and acid maleate, acid oxalate, acid sulphate, glucose phosphate, tartrate and acid tartrate.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/IT 98/00318

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K31/22

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	L.G.SPAGNOLI ET AL.: "Propionyl-L-carnitine prevents the progression of atherosclerotic lesions in aged hyperlipemic rabbits" ATHEROSCLEROSIS, vol. 114, no. 1, 1995, pages 29-44, XP002101594 cited in the application	1,2
Y	see abstract see page 29, right-hand column see page 40, left-hand column --- -/--	3-6



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

29 April 1999

Date of mailing of the international search report

18/05/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Tzschoppe, D

INTERNATIONAL SEARCH REPORT

Intern. Application No

PCT/IT 98/00318

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	A.ORLANDI ET AL.: "Propionyl-L-carnitine exerts a specific control on serum triglyceride levels and plaque progression in hypercholesteremic aged rabbits" AGE, vol. 16, no. 3, 1993, page 117 XP002101595	1,2
Y	see abstract	3-6
X	--- EP 0 681 839 A (KURATSUNE HIROHIKO ;KITANI TERUO (JP)) 15 November 1995 see column 5, line 9 - line 30 see column 6, line 34 - line 47; claims 1,4	1,3,6
X	--- EP 0 793 962 A (SIGMA TAU IND FARMACEUTI) 10 September 1997 see claims 1,6	1,2,6
X	--- US 4 343 816 A (CAVAZZA CLAUDIO) 10 August 1982	1,2
Y	see column 1, line 5 - line 17; claims 1,2	3-6
Y	--- US 5 786 326 A (HORWITZ LAWRENCE D) 28 July 1998 see abstract	3-6

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/IT 98/00318

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0681839 A	15-11-1995	JP 8026987 A US 5576348 A	30-01-1996 19-11-1996
EP 0793962 A	10-09-1997	CA 2198833 A JP 9323927 A US 5811457 A	04-09-1997 16-12-1997 22-09-1998
US 4343816 A	10-08-1982	AU 535518 B AU 5526780 A AU 535565 B AU 5526880 A BE 881680 A BE 881681 A CH 642848 A CH 641673 A DE 2911670 A DE 3005208 A FR 2448347 A FR 2448348 A GB 2043443 A GB 2046593 A JP 1609921 C JP 2033015 B JP 55136227 A JP 1609922 C JP 2033014 B JP 55136223 A NL 8000741 A NL 8000768 A US 4346107 A	29-03-1984 21-08-1980 29-03-1984 21-08-1980 30-05-1980 30-05-1980 15-05-1984 15-03-1984 14-08-1980 21-08-1980 05-09-1980 05-09-1980 08-10-1980 19-11-1980 15-07-1991 25-07-1990 23-10-1980 15-07-1991 25-07-1990 23-10-1980 14-08-1980 14-08-1980 24-08-1982
US 5786326 A	28-07-1998	US 5721209 A AU 6322898 A WO 9834619 A AU 699916 B AU 4674496 A CA 2185661 A CN 1145588 A CZ 9602889 A EP 0754044 A HU 9603036 A WO 9623502 A PL 316612 A US 5837677 A	24-02-1998 26-08-1998 13-08-1998 17-12-1998 21-08-1996 08-08-1996 19-03-1997 16-04-1997 22-01-1997 28-05-1997 08-08-1996 20-01-1997 17-11-1998

